AN INFRARED IMAGE ACQUISITION AND ANALYSIS METHOD FOR QUANTIFYING OPTICAL RESPONSES TO CHEMICAL AGENT VAPOR EXPOSURE

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ABSTRACT

A method was developed employing an infrared (IR) light source, IR capable video camera, digital processor and image acquisition/analysis software for quantifying pupil constriction resulting from nerve agent vapor exposure. Image acquisition and analysis routines were developed to capture images and automate pupil area measurements or manually analyze images based upon operator-selected parameters. The latter option is useful in cases where the pupil image is partially obstructed or pupil shape is other than circular. The method described here allows for remote/non-invasive assessment of the level as well as the time course of onset and duration of pupil constriction.

INTRODUCTION

One of the first noticeable effects of nerve agent vapor exposure is pupil constriction, otherwise known as miosis. A subject with miosis may have dimmed vision, resulting in difficulty seeing under low-light conditions.   Traditional methods of measuring miosis include: a microscope equipped with a light and reticule eyepiece, a pupillometer card for comparison of a subjects' pupil diameter with pre-measured pupil diameters, and infrared (IR) video systems using various techniques to determine pupil size. Some traditional IR video systems have used time based measurements (Murray and Loughnane, 1980) or specialized video array equipment (Jones et al., 1992).   These traditional IR methods are inadequate for area measurements of partially obstructed pupils, because of their inability to account for the non-visible areas of the pupil. Currently our lab utilizes a system whereby IR light (880nm) reflects from the retina back through the pupil producing a bright pupil surrounded by a dark iris (Fig. 1).   This IR light produces a well-defined image of the pupil for non-invasive measurement. This paper describes two methods developed to quantify the pupil sizes of rats and swine. The first method is an automated image acquisition and quantitation program. It saves images and measures the pupil by using image contrast to isolate the pupil from the iris. This program is best suited for measuring pupil area of pupils that are circular. The second method is useful for measuring pupils that are not circular in shape and/or partially obstructed by eyelids etc. (Fig. 1). It accounts for the non-visible area of the pupil by basing the measurements on geometric calculations.

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Without IR light

With IR light

Fig. 1   The effects of IR lighting on the pupil of a swine.
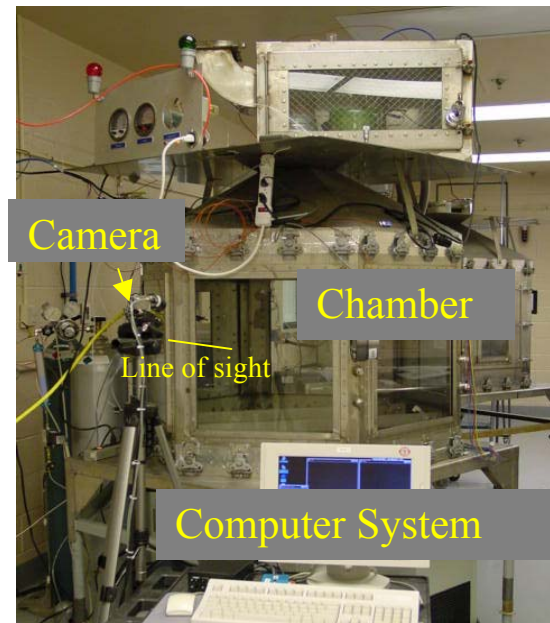

Camera

Chamber

Line of sight

Computer System

Fig. 2   Vapor chamber and imaging system.

METHODS

IMAGING EQUIPMENT AND COMMERCIAL SOFTWARE

National Instruments (Austin, TX.) manufactured the Labview and IMAQ software used for writing the automated and manual image analysis programs. They also produced the image acquisition computer card (PCI-1411) that receives the video signal input for the computer.

Data Science Automation (Canonsburg, PA.) was the supplier for the Sony CCD black and white video camera (XC-ST50), video camera power supply (CD 700), video camera 75mm/ F2.7 lens (LMV7527), tripod adapter (VCT-ST701), and the (2)100 candle power IR spotlights (SL2420-880100XL24VOLT). Figure 2 shows the entire experimental setup to include the camera, IR Lights, computer, and exposure chamber.

AUTOMATED PROGRAM

The automated program performs three main functions; the first function is to capture the video image and save it as a jpg image file using the animal number and image number to create a unique file name. This image is not altered but saved in a raw form for future use. The second function filters a copy of the image to isolate the pupil from the remainder of the image and measure it. The third function is to time-stamp and transfer image information, such as pupil size and animal number, into a spreadsheet format.

The second function is the most complex of the three functions because it requires many filtering steps in the isolation process. The raw starting image shown in Figure 3 provides a good sample image to demonstrate each of these steps.

2

Filtered Image

Any computer-generated image consists of pixels with differing colors/densities which create the contrast between objects in the image. In the program we are using, there are 256 different colors/densities of gray, ranging from white to black, that can be assigned to each pixel.

In any digital camera there are many small light sensors or pixels located behind the lens. The images are reflected upon these light sensors and converted to a digital signal. All the image details that are reflected onto a single sensor are averaged. This averaging creates a soft focus effect that is sharpened by the computers use of a convolution algorithm that adjusts the pixel densities to redefine the image details (Figs. 3 & 4). The convolution algorithm works by using the density values of neighboring pixels to better define the pixel density separation or contrast of the image.

Figure. 3 Raw image.

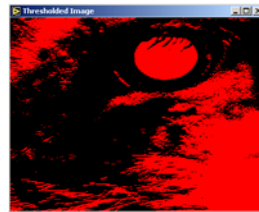Figure. 4 First filter using convolution algorithm resulting in sharper image.

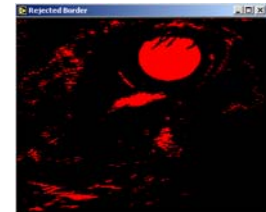Figure. 5 Results of a threshold limits separation.

Figure. 6 Image after border rejection.

Threshold Limit

The threshold limit value allows separation of the image into two colors, red or black (Fig. 5). All pixels with a density at or above the preset threshold limit are masked in red. The red masked section representing the pupil is then used for area calculations. All pixels below the preset threshold limit are masked in black. This converts the image from 256 densities to a red and black image with the pupil separated from the iris.

Border Rejection

The filtering step that removes the major portion of extraneous red masked areas of an image is known as border rejection (Figs. 5 & 6). This step eliminates any red masked areas that touch the border of the framed image. An example of border rejection would be a pupil masked in red, surrounded by an iris and eyelids masked in black, with neighboring fur masked in red. The red masked fur would be eliminated because it touches the border of the image frame. Therefore, it is imperative that no part of the pupil touches the image frame. The resulting image will now have only small red masked items and imperfections to eliminate from inclusion in area calculations.

Closing Function

The closing function eliminates imperfections of the pupil image caused by items such as eyelashes or foreign objects, such as bedding (Figs. 7 & 8). Any boundary between a red masked area and a black area forms a perimeter that is manipulated by the closing function. The closing function fills the imperfections in the perimeters by expanding all red borders of the masked areas to a preset limit. This is known as dilation. Following dilation, the masked area borders are reduced to the same preset limit. This

returns the red masked items to their original dimensions without imperfections. This is known as erosion. The entire process of dilating then eroding is known as closing.

Dilation   *Erosion*

Figure. 7   Illustration of closing function.

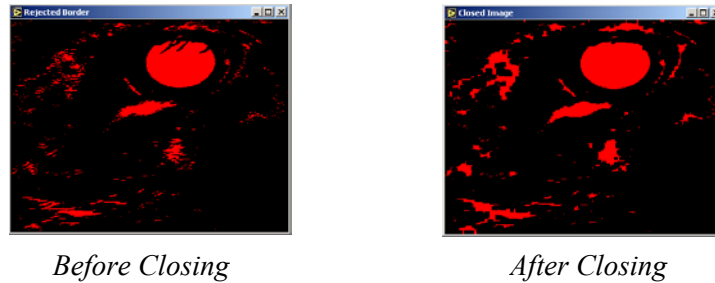*Before Closing*   *After Closing*

Figure. 8   Shows the elimination of the eyelashes from the pupil by the closing function.

Particle Filtering

After the imperfections have been eliminated, the computer must isolate the pupil and eliminate other red masked objects in the image (Fig. 9). Particle filtering removes all red masked items that are too angular in shape to be part of an ellipse or circle.

Object Measurement

For circular pupils, the automated software measures the diameter of the circular red masked object (pupil) and uses it to calculate the pupil area (Fig. 10). For elliptical pupils, the manual program is used to calculate the area.
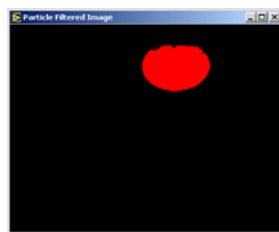

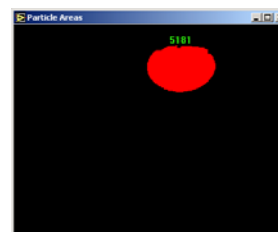Figure. 9 Shows the results of the particle filter.

Figure. 10 The pupil area displayed with image.

MANUAL PROGRAM

The manual program is designed to measure objects that are beyond the scope of the automated imaging program, such as partially obscured ellipses.   The manual program is not designed to be used in real time. The images to be measured are jpg image files saved prior to any filtration from the automated program.   Unlike the automated program the operator can adjust the image to separate and select the pupil for measurement. These adjustments are described below.

Image Controls

With the manual program the operator can use slide controls to adjust contrast, brightness, and gamma to separate the pupil from the iris (Fig. 11). The gamma is a brightness adjustment that does not adjust all pixels equally, but adjusts the mid range disproportional to the high and low density ranges. This allows for greater image separation within the high or low density ranges.
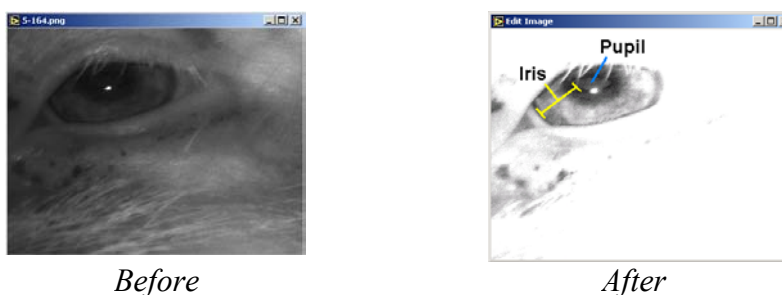

Before   *After*

Figure. 11   The effects of image controls.

Tolerance

The operator can select a range of values from a density scale spanning 0-255 possible image density values (tolerance). The operator clicks on a pixel in the pupil to be measured. The computer then draws a border around all adjacent pixels that are within the selected density range. For example, a tolerance range of +/- 12 and a selected pixel with a density value of 115 would result in a border being drawn around pixels with density values ranging from 103 to 127. The images shown below demonstrate the drawing of a tolerance border (Fig. 12).

Figure. 12   Diagram illustrating how the tolerance function
determines the border location around the pupil.

Calculations

        Three points alone the edge of the pupil image are needed to make the area calculation. The three points are the two horizontal extremes and one vertical extreme. If the bottom extreme point is obscured, the top extreme point can be used. In calculating the area of a partially obstructed ellipse, the center of the pupil was determined by finding the midpoint of a line between the two horizontal extremes. From the center of the pupil, the lengths of the horizontal and a vertical radii were determined and used to calculate the area of the ellipse (Fig. 13).
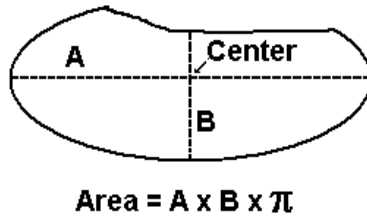

Figure. 13   Illustration of calculations made for determining the area of the ellipse.

Precision of Repeated Pupil Measurements

        The pupil areas of four different pupil images depicting common pupil conditions were each measured fifty times (Table 1). The standard errors in each of the four pupils under different conditions were less than 2% despite the difference in areas and intensities of the respective pupils.

Table 1. Mean and standard errors of images illustrating four typical pupil conditions each measured fifty times.

Condition	Pupil Mean	S.E.
Normal	7009.68	<1%
Partially obstructed top	7311.27	<1%
Partially obstructed bottom	5037.07	<1%
miosis	1254.07	1.2%

CONCLUSIONS

        The IR system and novel software described above allowed for the collection and quantification of data under low-light conditions. This system offers a non-invasive approach to continuous monitoring of subjects at a minimum distance of 40 inches. Though IR photographic systems have been previously employed in other studies (Murray and Loughnane, 1980)(Jones et al., 1992), to our knowledge none

have been able to accurately measure partially obstructed pupils. Due to its' versatility, this IR photographic system could be applied to other areas such as the measurement of evaporation rates of droplets from surfaces, the counting and measurement of particle sizes and measurement of growth rates of bacteria, molds, etc.

REFERENCES

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2. Jones D. P., Harris S. J. and Scicinski S. R. (1992) Pupillometer for clinical applications using dual 256-element linear CCD arrays.